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MICA polymorphism in a northern Chinese Han population: The identification of a new MICA allele, MICA*059

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ABSTRACT

The major histocompatibility complex class I chain-related gene A (MICA) in humans, located 46 kb centromeric to human leukocyte antigen (HLA)-B, is highly polymorphic. In addition to its primary role in immune surveillance, recent data highlight the importance of MICA in organ transplantation and in susceptibility to some diseases. In this study, 104 healthy, unrelated Han subjects recruited from central Inner Mongolia Autonomous Region, northern China, were investigated by sequence-based typing and fragment analysis for MICA allelic variation, MICA-HLA-B linkage disequilibrium, and HLA-A-Cw-B-MICA haplotypic diversity. Nineteen MICA alleles were observed, the most frequent of which were MICA*00801, MICA*010, MICA*00201, MICA*00901, and MICA*045, with gene frequencies of 23.08%, 18.75%, 12.02%, 12.02%, and 8.17%, respectively. The peculiarity in HLA-B-MICA haplotypic configurations was also uncovered. In particular, there was a clear-cut dichotomy between MICA*00801 and MICA*045 in their linkage to members of HLA-B*13 lineage, which was frequently represented in this population. A new MICA allele, MICA*059, was identified, which appeared to be evolutionarily linked to MICA*045. Haplotype HLA-A*30-Cw*06-B*1302-MICA*00801, previously not reported in other populations, was found with a frequency of 8.65% in this population. Our results provide new data about MICA genetic polymorphism in Chinese Han populations, which will form the basis for future studies of the potential role of MICA in allogeneic organ transplantation and disease susceptibility in related ethnic groups.

1. Introduction

Major histocompatibility complex class I chain-related gene A (MICA), which is located 46 kb centromeric to human leukocyte antigen gene B (HLA-B), is highly polymorphic with 67 MICA alleles being officially named (http://hla.alleles.org/data/txt/mica_nuc.txt). MICA gene is composed of 6 exons that encode the leader peptide, 3 extracellular domains (α1, α2, and α3), a transmembrane segment, and a carboxy-terminal cytoplasmic tail, respectively [1]. MICA molecule binds with NKG2D/DAP10 [2], an activating immunoreceptor complex expressed on natural killer cells, $\gamma\delta$T cells and CD8$^+$ $\alpha\beta$T cells [3]. MICA triggers the cytolysis of virus infected or transformed cells mediated by NKG2D-bearing cells [3] and provides co-stimulatory signal for CD8$^+$ $\alpha\beta$ T-cell activation in pathogen-specific immune response [4]; MICA polymorphisms have been shown to modulate binding affinities with NKG2D receptor [5]. In addition to the role in immune surveillance, recent data highlight the importance of MICA/MICB molecules in organ transplantation [6,7] and in HLA-linked disease association [8,9].

Data available indicate differential distributions of MICA alleles and MICA allele-containing HLA haplotypes among human populations [10–18]. Han ethnic group represents about 94% of the population in China and is divided into 2 genetically differentiated subgroups, northern Han and southern Han, which are separated approximately by the Yangtze river [19–21].

Inner Mongolia, China's northern border autonomous region, is the third largest subdivision of China covering about 12.3% of the country's territory. Han ethnic group constitutes the largest ethnic group and comprises about 79% (approximately 18 million) of the population in this region; Mongols are the second largest ethnic group comprising about 17% (approximately 4 million) of the population. There are also other ethnic groups in this region. According to the historical records, settlement of Han Chinese in central and western Inner Mongolia, mainly by inland farmers from neighboring northern Provinces, probably began during the Warring States Period (770-221 BC). The most recent episode of Han Chinese migration into this region occurred in the early 18th century during the Qing Dynasty, and continued into the 20th century.

Presently, there is a lack of information regarding MICA genetic variation in northern Chinese Han populations. In this study, 104 healthy, unrelated subjects of Han ethnicity recruited from central Inner Mongolia Autonomous Region were investigated for MICA allelic variation by sequence-based typing (SBT) and fragment analysis. We also report a novel MICA allele identified in this population.

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2. Materials and methods

2.1. Subjects

The unrelated panel consisted of 104 individuals of Han ethnicity recruited from Baotou Prefecture, central Inner Mongolia Autonomous Region [22]. Full Han ethnicity through both maternal and paternal grandparents and familial residence in the area for the last 3 generations was claimed by all participants. The study group comprised 59.62% women (62/104) and 40.38% men (42/104).

Among them were four parental samples derived from two trio families, which were studied for MICA exons 2–4, HLA-A, Cw, and B loci as quality control of the MICA-SBT protocol. A sibling of the individual bearing a novel MICA allele was also examined for MICA exons 2-4.

A blood sample was taken with each person's informed consent. All protocols were approved by the Institutional Review Committee of local authorities.

2.2. MICA typing

Polymerase chain reaction (PCR) primers previously used for PCR–sequence specific oligonucleotide probing of MICA gene [12,14,23] were adopted for the current study. The sequence and location of each primer, as well as the nucleotide position of each exon are given in Table 1. Exons 2, 3, and 4 were amplified separately in a 50-μl volume; PCR conditions were identical to the previous reports [12,14]. PCR product was purified using the EZ Spin Column DNA Gel extraction kit (Sangon, Shanghai, China), sequenced using exon-specific PCR primers and the ABI PRISM BigDye Terminator v3.1 sequencing kit on an ABI PRISM 3730 DNA sequencer (Applied Biosystems, Foster City, CA). For the MICA alleles encountered only once in our sample panel, sequencing of the exon of interest or exons 2, 3, and 4 was repeated in at least 1 additional independent PCR reaction. Confirmatory sequencing was also carried out for selected samples that deviated from expected HLA-B-MICA associations. The sequence chromatograms were analyzed using program Chromas. Raw sequence signals were manually reviewed, the haplotypic phase of single nucleotide polymorphisms in each exonic segment was determined on the basis of MICA gene sequence database (http://hla.alleles.org/data/txt/mica_nuc.txt), blastn analysis was then performed for both DNA stretches against the EMBL Release database. MICA allele assignment was based on the integrated information of exons 2–5.

Fluorescent PCR–fragment analysis was used to analyze the (GCT)n microsatellite at exon 5 of MICA gene (abbreviated as MICA-STR) [12,14,24], detection of MICA gene deletion (MICA*Del) was carried out by PCR–sequence specific priming (PCR-SSP) as previously described [24].

The Guanine at nucleotide position 820 in exon 4 discriminates MICA*045 from MICA*007 lineage (http://hla.alleles.org/data/txt/mica_nuc.txt). Considering that (a) MICA*045 allele showed a G peak of decreased intensity and sharpness at nucleotide position 820 in exon 4 in some heterozygotes; and (b) several MICA alleles, including MICA*008 lineage and MICA*010, both of which were prevalent in this population, possess a Guanine at nucleotide position 821 in exon 4 resulting in a shoulder G peak at position 820, all MICA*045 alleles were further confirmed using PCR-SSP following sequence-based typing. The PCR primers were adopted from the report by Collins *et al.* [25]. Each PCR reaction included an internal control and was carried out using the protocol previously described for the detection of MICA gene deletion [24] with minor modifications.

2.3. Cloning of exon 2 of a novel MICA allele

During MICA-SBT process, a new sequence motif differing from that of MICA*045 by a single nucleotide polymorphism from T to G at position 146 in exon 2 was observed in an individual and also detected in 1 sibling. PCR product of MICA exon 2 derived from this individual was therefore cloned using a TA cloning kit (Takara, Dalian, China). The inserts of 20 randomly selected recombinant plasmids were sequenced in both directions.

2.4. HLA typing

HLA-A, Cw, and B data by generic PCR-SSP have been reported in our previous study [22]. In the present study, HLA-B*13 allelic typing was achieved by PCR-SSP using a commercial subtyping kit (Invitrogen, Brown Deer, WI).

2.5. Statistical analysis

The program ARLEQUIN 3.11 (http://cmpg.unibe.ch/software/arlequin3/) [26] was used to analyze the data, including Hardy–Weinberg equilibrium (HWE) test, multilocus haplotype inference, and pairwise global linkage disequilibrium (LD) analysis that aims to characterize the total association between HLA-B and MICA rather than a specific LD between 2 particular alleles at these 2 loci. Maximum likelihood haplotype frequencies were estimated using the expectation-maximization (EM) algorithm, as implemented in the program ARLEQUIN 3.11. The classic coefficients of LD, Δ, and of normalized LD, Δrel, were computed for each individual haplotype as described previously [22,27]. Fisher exact test was performed to determine the significance of Δ. The LD analysis was restricted to HLA-B-MICA haplotypes with observed frequencies $>1.5\%$. Statistical significance was defined at the 5% level after the p value was adjusted using Bonferroni's correction, that is, by multiplying the p value by the number of independent comparisons performed.

3. Results

There were no missing genotypic data.

3.1. MICA and MICA-STR HWE tests

The MICA allelic distributions were consistent with Hardy–Weinberg proportions ($p = 0.1372$), whereas the MICA-STR locus showed a trend toward deviation from HWE ($p = 0.0434$, $p_c = 0.0868$).

3.2. MICA and MICA-STR allele frequencies

The MICA and MICA-STR allele frequencies are summarized in Table 2. Nineteen MICA and 6 MICA-STR alleles were detected in

Table 1
Primers used for sequence-based typing of exons 2, 3and 4 of MICA gene[a]

Exon	Primer sequence (5′-3′)	Primer location	Length of exon (bp)	Exon location
2	Forward: TCTTgTCCCTTTgCCCgTgTgCAT	6826-6849	255	6950-7204
	Reverse: CCCCCATTCCTCACCCCCAgCCTg	7301-7324		
3	Forward: TgggggAgggCCAgggAggCgTAC	7378-7401	288	7479-7766
	Reverse: CgATgTgCCAACAggAAATgCCTT	7866-7889		
4	Forward: CAgACTTgCAggTCAggggTCCCg	8227-8250	279	8354-8632
	Reverse: CAATgACTCTgAAgCACCAgCACT	8736-8759		

[a]All nucleotide positions were based on MICA consensus sequence (Genbank accession number: X92841).